Characterization and Structure by NMR and FTIR Spectroscopy, and Molecular Modeling of Chromium(III) Picolinate and Nicotinate Complexes Utilized for Nutritional Supplementation

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Abstract

Chromium picolinate (CrPic) and chromium nicotinate preparations (CrNic1, CrNic2, CrNic3) were investigated with ^{1}H and ^{13}C NMR, FTIR, and molecular modeling. CrPic is crystalline and bidentately coordinated. Cr–PicA bonding broadens the NMR signal or shifts it so far downfield that it is not detectable.

All CrNic preparations are noncrystalline, and results provide no evidence that nicotinic acid (NicA) is O-coordinated to Cr. The complex colors may be due to O-coordination with H_2O and/or OH, not NicA. ^{1}H NMR spectra of CrNic1 have two sets of peaks. One set has a significant δ with respect to NicA, indicating that NicA is more strongly associated with Cr. CrNic1 ^{13}C data show small, uniform δ with respect to NicA, indicating that strong localized Cr–COOH bonding is unlikely. The magnitude of δ, ^{13}C, and ^{1}H exchange data suggests that limited Cr–N bonding may occur in CrNic1. CrNic2 and CrNic3 show little difference from NicA spectra.

FTIR spectra of all CrNic complexes, but not CrPic, PicA, or NicA, show bound OH and/or H_2O. CrNic complexes are probably olates, with Cr and NicA OH-polymerized. CrPic exchanges with CrNic1 in DMSO. This exchange may provide a mechanism for the absorption and active transport of Cr in biological systems.
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Introduction

The essentiality of trace amounts of chromium(III) is well known [1, 2]. In an active form, Cr functions to improve insulin biological activity. Cr supplementation in humans and animals reduces symptoms of Type II diabetes and hypoglycemia. Cr also positively affects plasma lipids resulting in increased high-density lipoprotein (HDL) and decreased total cholesterol and triglycerides. Cr supplementation increases lean body mass in swine [3, 4] and in humans [5, 6], but some human studies are more equivocal [7].

Several Cr(III) organic complexes have been shown to have significantly higher absorption and tissue incorporation activity than inorganic salts such as chromic chloride ($CrCl_3 \cdot xH_2O$) [8, 9]. Research on Cr nutrition has focused primarily on Cr complexed with organic acids, such as nicotinate and picolinate, or Cr organic acid–amino acid complexes, with $CrCl_3$ used as the standard.

The realization that a nutrition supplement can improve insulin function, cardiovascular disease risk factors, and potentially aid body fat loss has led to the dramatic success of commercial Cr nutritional supplements. The two most common in the market are "chromium picolinate" (CrPic) and "chromium polynicotinate" (CrNic). Despite widespread use of these products in both research and retail arenas, neither has been fully chemically characterized. Stearns and Armstrong [10] reported the X-ray diffraction structures of mono- and binuclear chromium picolinate H_2O complexes. $Cr(Pic)_3 \cdot H_2O$ was shown to be a red monoclinic (space group C2/c) crystal, with bidentate meridional ligand geometry. In contrast, CrNic has not been shown to be a crystalline compound.

Research on the chemical structure of biologically active Cr was initiated with the identification of an organic form of Cr from brewer's yeast [11]. Gonzalez-Vergara et al. [12] synthesized a trinuclear Cr(III) complex identified as $Na[Cr_3O(nicH)_6(H_2O)_3][ClO_4]_8 \cdot nicH \cdot 6H_2O$, where nicH = pyridyl protonated nicotinic acid. The complex consisted of emerald green crystals, slightly soluble in H_2O at neutral pH. Nicotinic acid (NicA) was reported to be bound to Cr via the carboxylate oxygen only. Green et al. [13] synthesized orange crystals of *trans*-$[Cr(1,3\text{-pn})_2(nic)_2]Cl \cdot 4H_2O$ (1,3-pn = 1,3 propanediamine) with

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deuterium-labeled NicA. Deuterium NMR spectroscopy indicated that the two *trans*-NicA were coordinated via the carboxyl group only, and the authors postulated that the complex was stabilized by the presence of the 1,3 propanediamine.

Cooper et al. [14] synthesized $Cr(II)(nic)_2(H_2O)_4$, a yellow crystalline solid, and $Cr(III)(nic)_2(H_2O)_3OH$, a blue solid (nic = nicotinic acid) from nicotinic acid solutions. Nicotinic acid in the Cr(II) complex was reported bonded only via the pyridine nitrogen; in the Cr(III) complex, they were bonded only via the carboxylate oxygen. A blue solution of Cr at pH 5 was the only compound that exhibited significant activity with the yeast glucose fermentation bioassay. Crystalline Cr(III) complexes with the amino acids alanine (ala), glycine (gly), histidine (his), proline (pro), glutamine (glu), and cysteine (cys) have also been synthesized [15, 16]. The amino acids were coordinated bidentately, and the complex colors varied from red to pink. Similar solid his, gly, EDTA, and leucine (leu) Cr(III) complexes were synthesized in our laboratory [9], and ranged in color from purple to peach. Attempts to synthesize Cr(III)–nic-gly and –cys complexes resulted in only complex green and blue solutions, respectively [15]. Cooper et al. [15] reported that only $Cr\text{–}glu_2(H_2O)_2^+$, Cr–nic–gly, and $Cr(gly)_n(H_2O)_{6-n}^{+3}$ solutions showed significant activity in the yeast glucose bioassay (pH 5.75). $Cr(III)nic_2gly_2cysglu$ (glu = glutamic acid) and $Cr(III)nic_2his_2$ solutions were also synthesized and investigated. $Cr(III)nic_2gly_2cysglu$ resulted in the highest Cr incorporation into rat kidneys as compared to nine Cr compounds tested. Cr concentration in rat livers was highest for CrPic, followed by $Cr(III)nic_2gly_2cysglu$ [9].

In general, Cr–N bonding produces yellow to red colors, and Cr–O bonding produces green to blue colors. Cr(III) binds strongly to OH and H_2O in aqueous solutions, often forming polymeric complexes [16]. Hydrated $Cr(OH)_n$ precipitate, with an emerald green color, is characteristic of basic aqueous solutions.

In this report, we have investigated the structures of CrPic (i.e., $Cr(Pic)_3$) and three preparations of CrNic with liquid phase NMR and solid phase Fourier transform infrared (FTIR) spectroscopy, and molecular modeling. We used three preparations of CrNic because of our concerns about variability in this product. For example: (1) no preparation of CrNic is crystalline, (2) each product investigated had a different color, (3) solubility characteristics among the products were dissimilar, and (4) preliminary results indicated that NMR spectra of various Cr complexes differed significantly.

Materials and Methods

Chromium Compounds

CrPic was synthesized according to U.S. Patent 4315927 (Nutrition 21, San Diego, CA). It is a ruby red crystalline compound (MW 418.3) sparingly soluble at neutral pH in H_2O (~ 1 ppm at 25°C) and soluble in methyl sulfoxide (DMSO) at > 6 mg/mL. The DMSO solution is clear red. Chromium nicotinate #1 (CrNic1) was synthesized from $CrCl_3$ and nicotinic acid in H_2O solution, and adjusted to pH 6.0 with conc. NaOH [9]. It is a dark green noncrystalline compound, soluble in both H_2O and DMSO at > 6 mg/mL. The DMSO solution is transparent green. CrNic2 was also synthesized in our laboratory according to U.S. Patent 4954492. It is a periwinkle blue–gray noncrystalline solid, soluble in H_2O and DMSO at < 1 mg/mL. A pale blue DMSO solution was used. CrNic3 was provided by InterHealth, Inc., Concord CA (ChromeMate® brand, U.S. Patents 4923855, 4954492, 5194615). It is a gray–violet powder, also soluble in H_2O and DMSO at < 1 mg/mL. A pale violet DMSO solution was used. No color changes or precipitation were observed upon dissolution in DMSO, and only transparent solutions were utilized.

NMR Spectroscopy

Solution ^1H and ^{13}C NMR spectra were recorded with a GE QE 300 MHz NMR spectrometer. All samples were dissolved in deuterated DMSO. Chemical shifts were referenced to the internal 0.0 ppm standard. A MacNMR v. 5.2 program with an Apple Macintosh Power Mac 8100/110 computer was used to process the data.

Mid-IR Spectroscopy

Chromium complexes and free acids were analyzed as powders by solid-state mid-infrared diffuse reflectance. A CO_2 free dry air purged Fourier transform IR spectrometer (FTS-60, BioRad, Inc., Cambridge, MA) was used at 4 cm^{-1} resolution (64 scans, KBr beam splitter, TGS detector). Background water absorption was insignificant, including the ν_3 region, which may interfere with absorption within the molecules. Spectra were recorded as relative absorption with respect to a KBr blank.

Molecular Mechanics

Global energy minimized molecular conformations for the model compounds $CrPic_3$, $CrNic_3$, $CrPic_2Nic$, and $CrPicNic_2$ were determined with the molecular mechanics program Sybyl 5.5 (Tripos Associates, St. Louis, MO) on a Sun Workstation (Sun, Inc., Mountain View, CA). Initially, the energy minimized Tripos force field structures for PicA and NicA were constructed. Minimization of torsional energy on the acid group resulted in both of these molecules having planar structures. After the step which minimized torsional energy, program calculations with Gasteiger-Marsili electrostatics were used to minimize steric energy. This gave preferred orientations for the COOH group with respect to N. These calculations were at room temperature, gas phase, and without significant field effects from neighboring molecules. In the next step, three-dimensional conformations consisting of three orthogonal pyridine acid molecules surrounding a +3 charge representing Cr(III) were constructed. Gasteiger-Marsili electrostatics were used to energy-minimize the entire Cr complexes. The +3 charge is assumed to be a spherical point charge, and the absolute energy calcula-

tions are considered reasonable approximations. The set of structures is internally consistent, and comparison of the relative stability of the various conformations within the set is valid.

Results

NMR Spectroscopy

The peak assignments and δ values for PicA and NicA are given in Tables 1 and 2 and Fig. 1. The COOH group positions in in Fig. 1 reflect the lowest energy conformations from the modeling.

CrPic. CrPic in DMSO gave no NMR signal for ^{1}H or ^{13}C at chemical shifts up to 25 ppm (^{1}H, Fig. 2a). This was probably due to strong bonding between PicA and Cr [10, 13]. After adding 0.1 M NaOD to the sample, CrPic was partially dissociated, and a spectrum characteristic of free PicA became evident (Fig. 2b). Figure 2c shows the spectrum of PicA in DMSO solution. The CrPic in NaOD solution does not show the splitting that free PicA does, due mainly to the presence of strongly paramagnetic Cr. PicA was also run and processed in DMSO plus NaOD, and showed splitting not present in Fig. 2c (data not shown). Addition of a strong base as well as some interaction of Cr with PicA also chemically shifted spectrum 2b with respect to 2c. The basic CrPic solution was not stable, and formed a clear solution and an emerald green gelatinous precipitate within 12 h.

CrNic1, ^{1}H. The ^{1}H spectra for CrNic1 and NicA are shown in Fig. 3. CrNic1 (Fig. 3a) showed two sets of peaks. Peaks 1, 2, 3, and 4 are more characteristic of free NicA; however, splitting has been lost, and there is a slight chemical shift downfield. Peaks 5, 6, 7 are very strongly shifted towards 0.0 ppm. Figure 3b is a spectrum of free NicA for comparison, and peaks 5, 6, 7 are absent. Figure 3c is an approximately 1:1 molar ratio CrNic1:NicA, showing the dilution effect of NicA on the spectrum, and clearly identifying the two groups of peaks. Integration of spectrum 3a showed that there are actually four peaks in the "complex" area: peaks 5, 6, 7 overlay a broad peak 8. These spectra indicate that at least two types of interactions between Cr and NicA were present in the CrNic1 complex.

Table 1. Proton NMR Spectral Peak Assignments*

Assignment	Multiplicity	δ (ppm)
^{1}H PicA, Proton No.		
1, ortho to N	doublet	8.71, 8.69
2 & 3, meta and para to COOH	quintuplet	8.05, 8.03, 8.01, 7.98, 7.95
4, ortho to COOH	triplet	7.64, 7.62, 7.60
^{1}H NicA Proton No.		
1, ortho to N and COOH	singlet	9.07
2, ortho to N	doublet	8.79, 8.77
3, para to N	triplet	8.31, 8.28, 8.25
4, meta to N and COOH	triplet	7.57, 7.54, 7.52

* Proton chemical shifts and peak assignments for free picolinic and nicotinic acid. ^{1}H peaks for chromium complexes are shifted significantly from these positions and/or broadened; however, the proton assignments, where appropriate, do not differ.

Table 2. Carbon 13 NMR Spectral Peak Assignments*

^{13}C NicA, CrNic1 Peak No.		NicA	CrNic1
1, COOH	singlet	165.91	166.01
2, ortho to N	singlet	152.89	153.09
3, ortho to N and COOH	singlet	149.90	150.01
4, para to N	singlet	136.54	136.71
5, meta to N, bonded to COOH	singlet	126.41	126.37
6, meta to N	singlet	123.42	123.63

* Carbon 13 chemical shifts and peak assignments for free nicotinic acid and CrNic1. ^{13}C chemical shifts do not differ greatly between the free acid and the complex, as is the case with the ^{1}H spectra.

CrNic2, ^{1}H. Figure 4b shows the CrNic2 spectrum. This spectrum shows diminished splitting as compared to NicA (Fig. 3b), but does not show the second set of peaks characteristic of complexing seen in CrNic1, shown again on 4a, the uppermost curve.

CrNic3, ^{1}H. Figure 4c shows the CrNic3 spectrum. CrNic3 also shows diminished splitting as compared to NicA, but the splitting is more recognizable than in CrNic2. The second set of peaks is also not observed. Even though the number of scans was increased, signal/noise for CrNic2 and CrNic3 is lower than that for CrNic1 and NicA because the former compounds were so insoluble.

CrNic1, ^{13}C. The ^{13}C spectrum for CrNic1 is shown in Fig. 5a (upper curve), and that of NicA in Fig. 5b. The

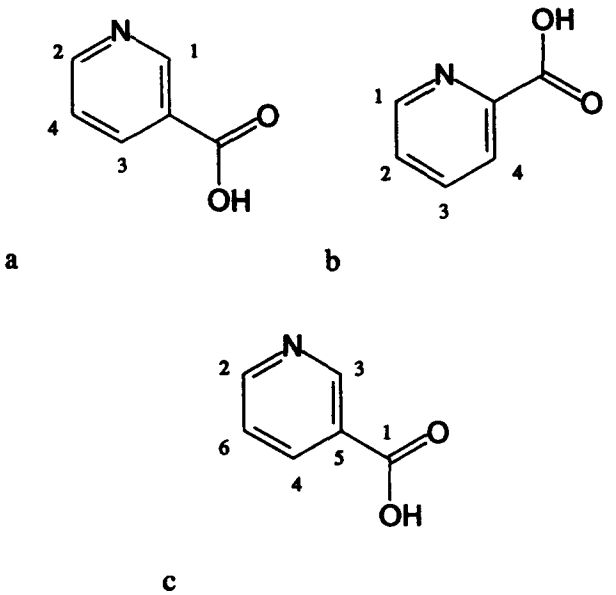

Figure 1. Proton assignments for nicotinic acid (a), picolinic acid (b), and carbon assignments for nicotinic acid (c). Refer to Table 1. COOH group positions in lowest energy conformations as calculated from the Sybyl program.

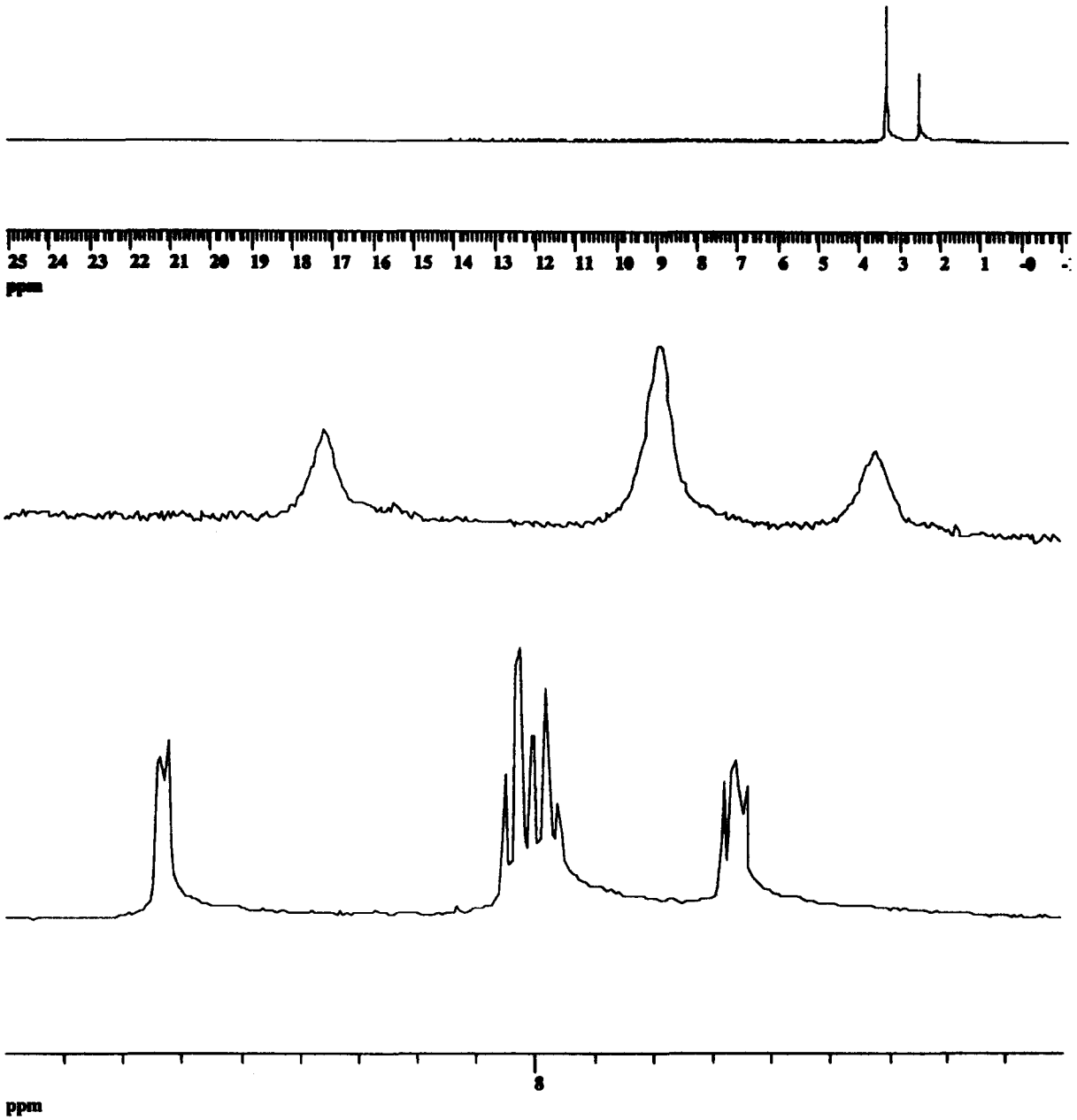

Figure 2. ^{1}H NMR spectra in DMSO, (a) Upper curve: CrPic gave no detectable signal to 25 ppm; peaks visible are DMSO and H_2O. (b) Middle curve: addition of 0.1 M NaOD chemically shifted and destabilized CrPic. (c) Lower curve: PicA.

^{13}C peaks in CrNicl show a uniform chemical shift of ~ 0.1 ppm downfield from the positions in NicA (Table 2), with the exception of C5, which is shifted 0.1 ppm upfield. This slight difference in δ may be explained by the fact that C5 has no H bound to it. Peak 1 represents the carbon in the acid group. Its chemical shift is no greater that of the carbons on the pyridine ring, indicating that strong, localized bonding between a carboxylic oxygen and Cr is unlikely.

CrPic and CrNicl Exchange, ^{1}H and ^{13}C. We investigated the exchange in solution between CrPic and CrNicl. The red CrPic and green CrNicl DMSO solutions were mixed in molar ratios from 4/12 CrPic to 7/12 CrPic. Solution colors were always intermediate between the two end members, and no precipitation occurred. For purposes of the mixtures, CrNicl·MW was calculated as $CrNic_3$. ^{1}H spectra for 4/12, 5/12, and 7/12 CrPic are given in Fig. 6. The CrNicl signal/noise decreases somewhat as CrPic increases, but this dilution effect is neither as dramatic nor as systematic as that seen in Fig. 3c, where NicA and CrNicl were mixed. The CrNicl spectral peaks broaden considerably, and change in relative intensity as compared to pure CrNicl (Fig. 3a). Peaks 3–7 also show splitting that is not apparent in CrNicl. However, the most significant features are the changes in peaks 1 and 2. These peaks become very broad, and dependent on the concentration, can com-

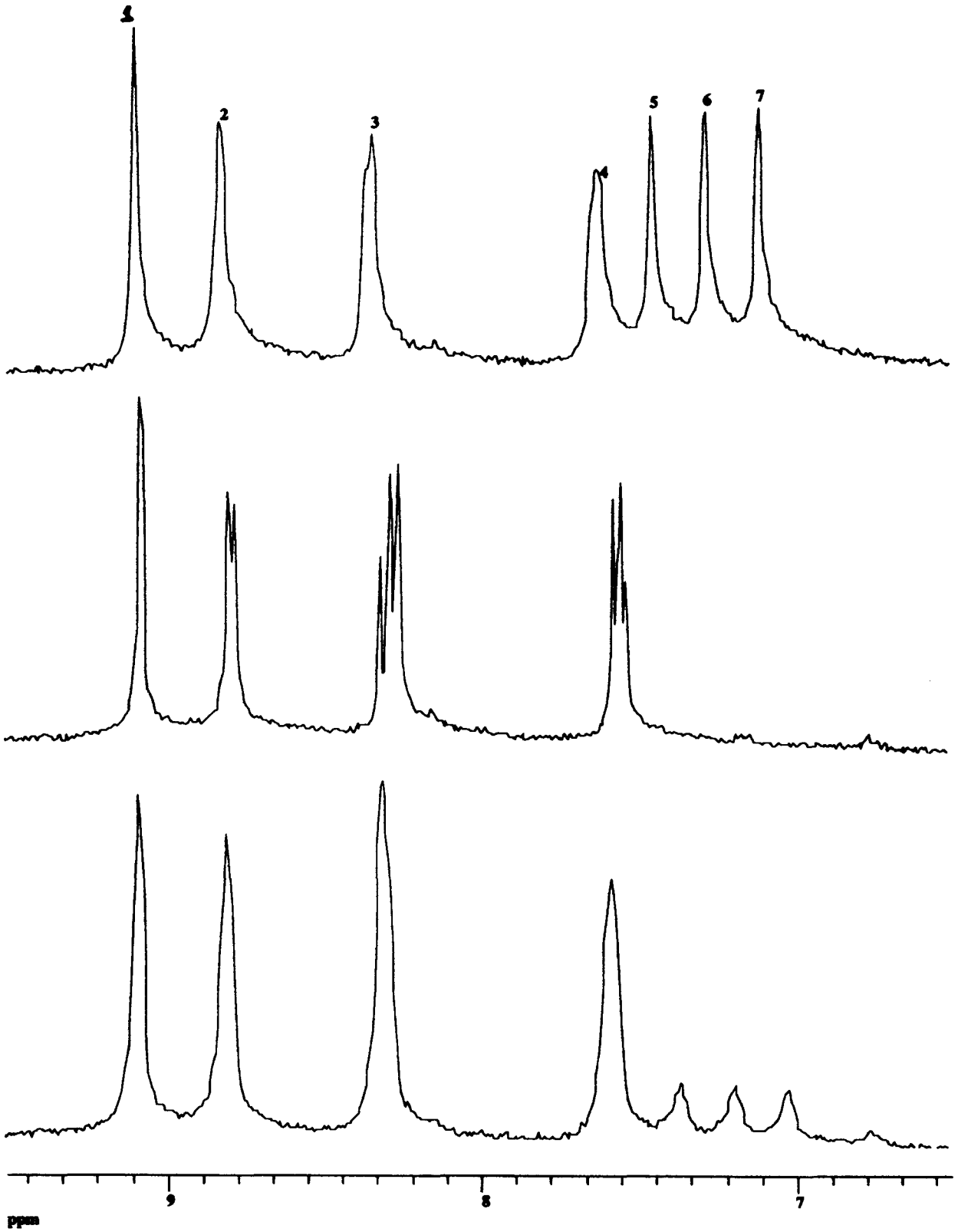

Figure 3. ^{1}H NMR spectra in DMSO, (a) Upper curve: CrNic1. (b) Middle curve: NicA. (c) Lower curve: CrNic1 and NicA in approximately 1:1 ratio. The CrNic1 peaks 5, 6, 7 in (a) are proportionally diluted by NicA.

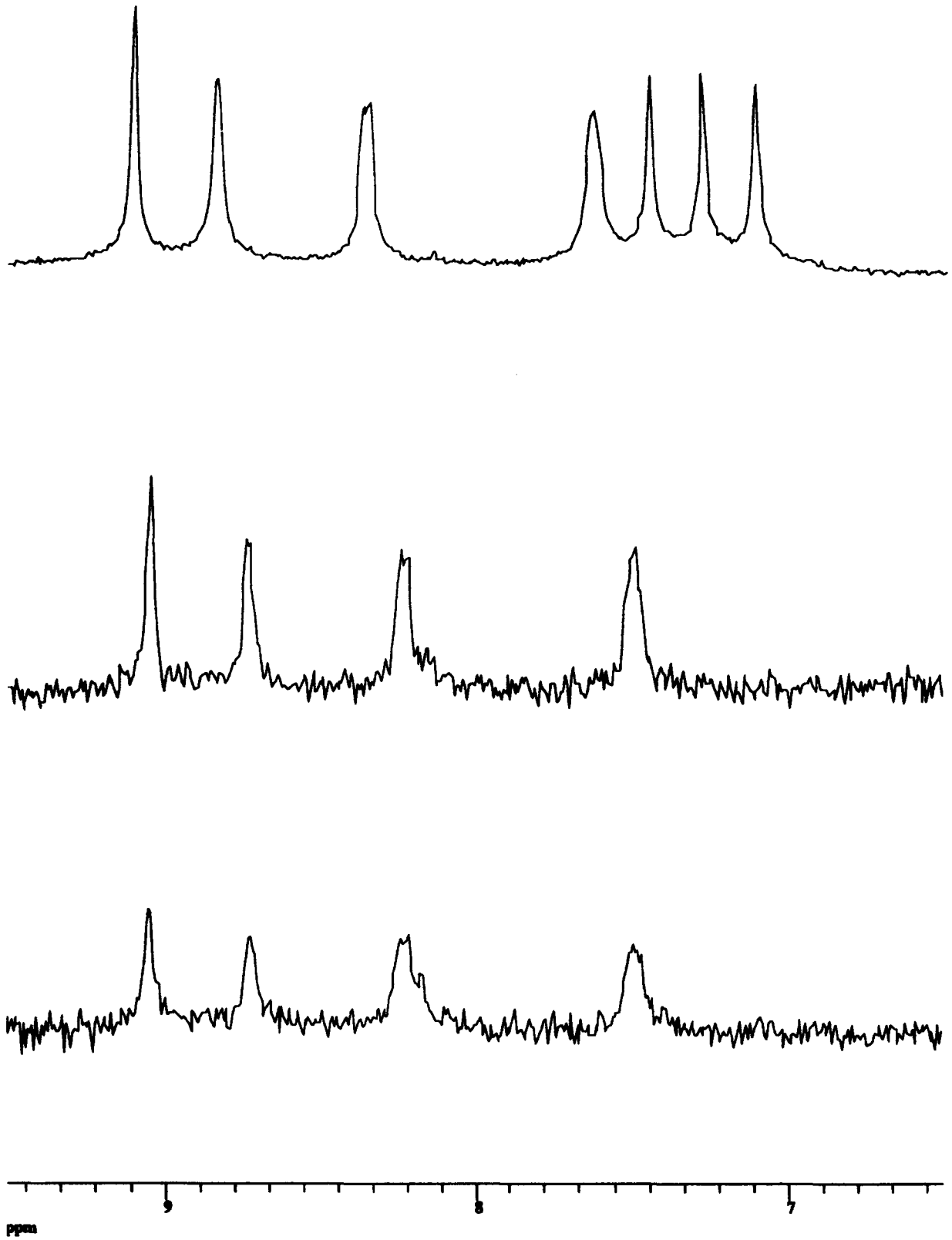

Figure 4. ^{1}H NMR spectra in DMSO. (b) Middle curve: CrNic2. (c) Lower curve: CrNic3. (a) Upper curve is CrNic1 (3a) for comparison.

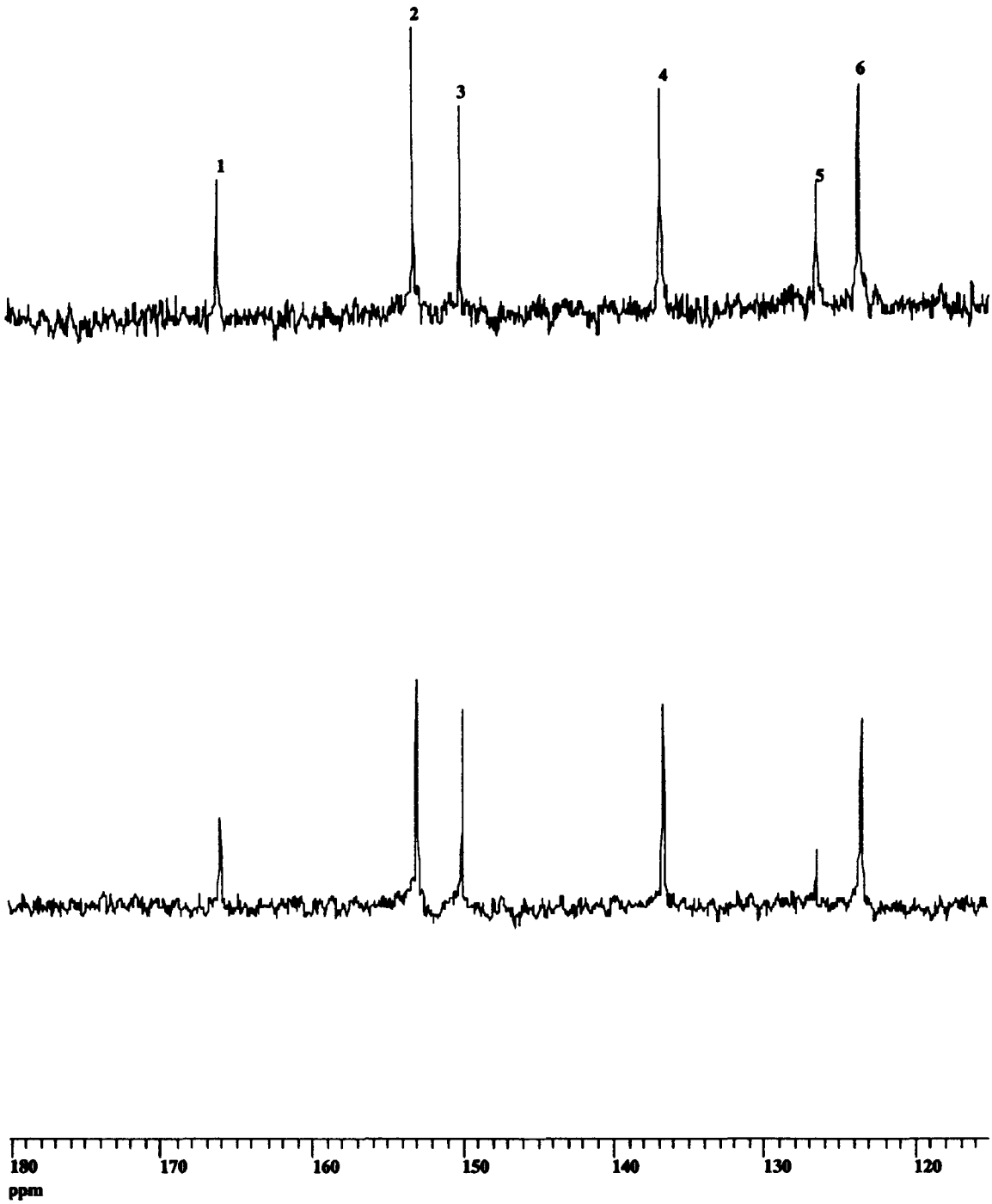

Figure 5. ^{13}C NMR spectra in DMSO. (a) Upper curve: CrNic1 120K scans. (b) Lower curve: NicA 10K scans.

pletely coalesce. The two formerly distinguishable CrNic1 proton environments are barely distinguishable on the time scale of the NMR experiment. These peaks represent the protons on either side of the N in the NicA pyridine ring. This type of behavior is not observed in Fig. 3c. ^{13}C spectra of CrPic/CrNic mixtures were also run and processed, but mixing in any amount of CrPic dramatically decreased signal/noise. Dilution of CrNic with more than 4/12 CrPic gave peaks barely distinguishable from noise even at 120,000 scans (data not shown). The ^{13}C 4/12 CrPic, 8/12 CrNic spectrum was shifted uniformly about 0.2 ppm upfield with respect to NicA. Mixing apparently did not affect the carbon environments much, and the CrPic served mostly to dilute and weaken the CrNic ^{13}C signal. A mixture of CrPic and NicA did not exhibit this behavior.

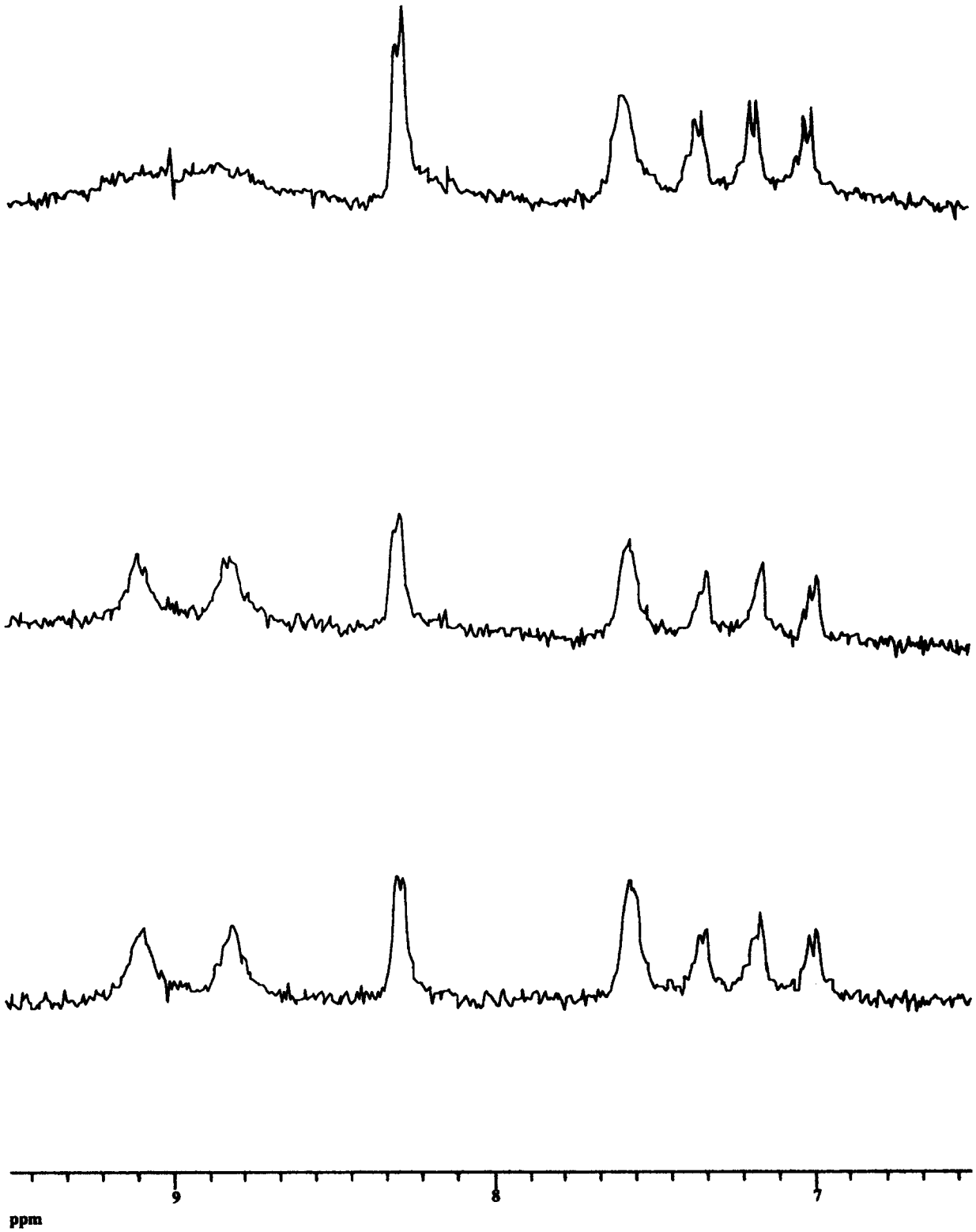

Figure 6. ^{1}H NMR spectra of mixed CrPic and CrNicl DMSO solutions. Starting from top, spectra represent 4/12, 5/12, and 7/12, CrPic, respectively. Peaks 1 and 2 (Fig. 3a) are specifically broadened by the mixing.

Molecular Structures

Stable structures for $CrPic_3$, $CrPic_2Nic$, $CrPicNic_2$, and $CrNic_3$ are given in Fig. 7, and steric energies for all molecular conformations are given in Table 3. $CrPic_3$ was the most stable conformation, and the structure was in basic agreement with that proposed by Stearns and Armstrong [10]. $CrPic_2Nic$ with the single NicA bonded to Cr via N was almost as stable as $CrPic_3$. Bonding of a single nicotinic acid via O was less stable. Similar results

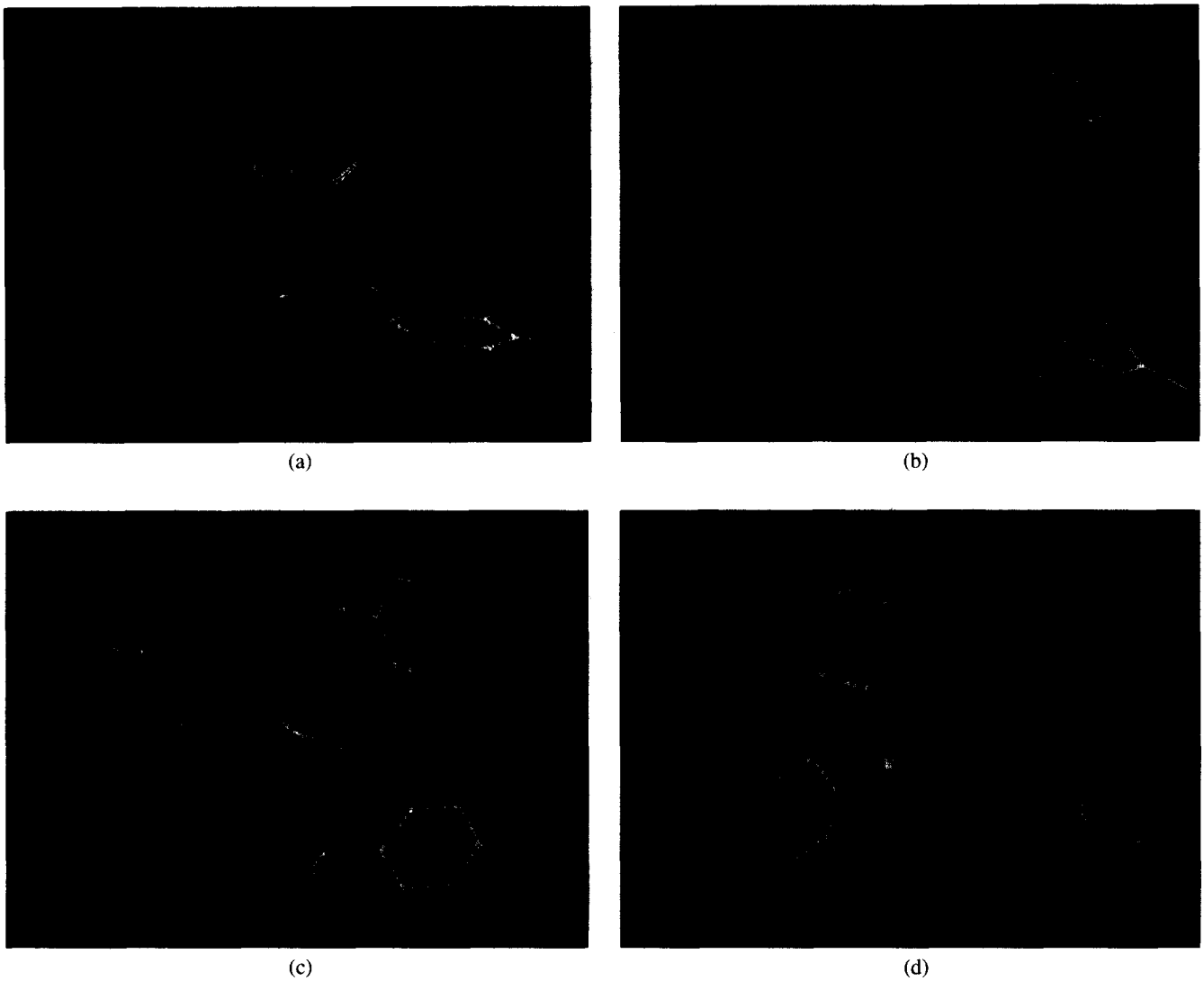

Figure 7. Three-dimensional energy-minimized molecular structures calculated and drawn with the Sybyl program. In all cases, Cr was represented initially by a symmetrical point charge, which adjusted to a nonsymmetrical shape during energy minimization. See Table 3 for molecular energies. (a) $CrPic_3$, Cr, and three picolinic acid molecules in the most stable conformation. (b) $CrPic_2Nic$, with the NicA pyridyl N oriented towards Cr. (c) $CrNic_2Pic$, NicA with the COOH group oriented towards Cr. This structure is less stable than (b). (d) $CrNic_3$, with the NicA N oriented towards Cr. Structures with 3 NicA with COOH oriented towards Cr were the most unstable, and are not shown.

were observed for $CrPicNic_2$ structures; however, energies were higher, and energy-minimized structures were less symmetrical. $CrNic_3$ with three NicA bonded via O was totally unstable; during energy minimization, the 3 + charge migrated far from the vicinity of the pyridine rings. Three NicA molecules bonded via N only were significantly more stable.

IR Spectroscopy

The solid-state IR diffuse reflectance spectra for CrPic and PicA are given in Fig. 8, and those for CrNic1, CrNic3, and NicA in Fig. 9. In each spectrum, the strong series of absorption bands centered around 1500 and 700 cm^{-1} are due to the pyridine rings. Broad absorption bands centered around 2500 cm^{-1} are due to carbonyl C–C = O bonds. The sharp peaks at 3100 cm^{-1} on PicA (8b) and NicA (9c) are due to the single OH on the carboxylic acid group. None of the CrNic complexes exhibits this sharp peak, but show broad absorption bands centered around 3000 cm^{-1}, a characteristic of numerous OH groups or hydrolyzed compounds. These peaks were not due to absorbed water because they were absent in the comparison PicA and NicA spectra. The strong absorption in CrPic (8a) from about 2900 to 3600 cm^{-1} is not completely assigned, and probably consists of overlaying peaks. Strong absorption bands in this region occur from NH and OH stretching vibrations [17]. The monomeric carboxylic acid OH stretch occurs from 3580 to 3500 cm^{-1}. Aromatic CH stretching occurs around 3100 cm^{-1}, but this is a weak absorption and cannot account for all absorption in this region. These

Table 3. Molecular Conformation Energies*

Molecule	Energy (kcal / mol)
$CrPic_3$	−109.6
$CrPic_2Nic$, NicA N bonded	−100.1
$CrPic_2Nic$, NicA O bonded	−85.7
$CrPicNic_2$, NicA N bonded	−89.8
$CrPicNic_2$, NicA O bonded	−61.5
$CrNic_3$, N bonded	−79.3
$CrNic_3$, O bonded	+32.5 (unstable)

* Conformation energies calculated from the Sybyl program. More negative values indicate greater stability; positive values indicate unstable structures. N bonding indicates nicotinic acid with nitrogen oriented towards Cr, and O bonding indicates nicotinic acid with COOH oriented towards Cr.

2900–3600 cm^{-1} absorption features are characteristic of the CrPic complex, and may reflect vibrations of OH–Cr–N bonds. CrNic2 was also run, but the spectrum was nearly identical to CrNic3, so it is not shown. The CrNic complexes were not significantly different in IR absorption, as opposed to great variation in their visible colors, solution behaviors, and NMR spectra.

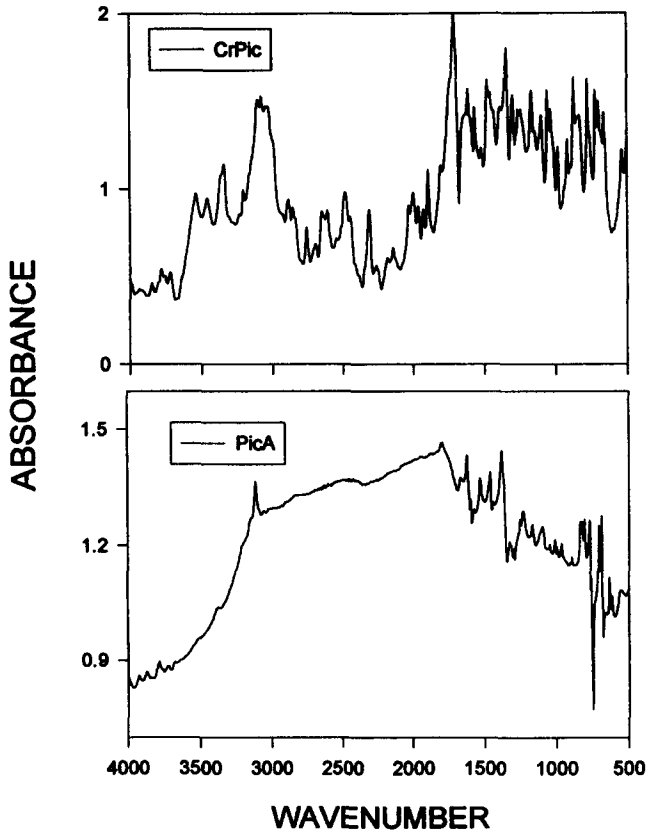

Figure 8. FTIR diffuse reflectance spectra. (a) Upper curve: CrPic. (b) Lower curve: PicA.

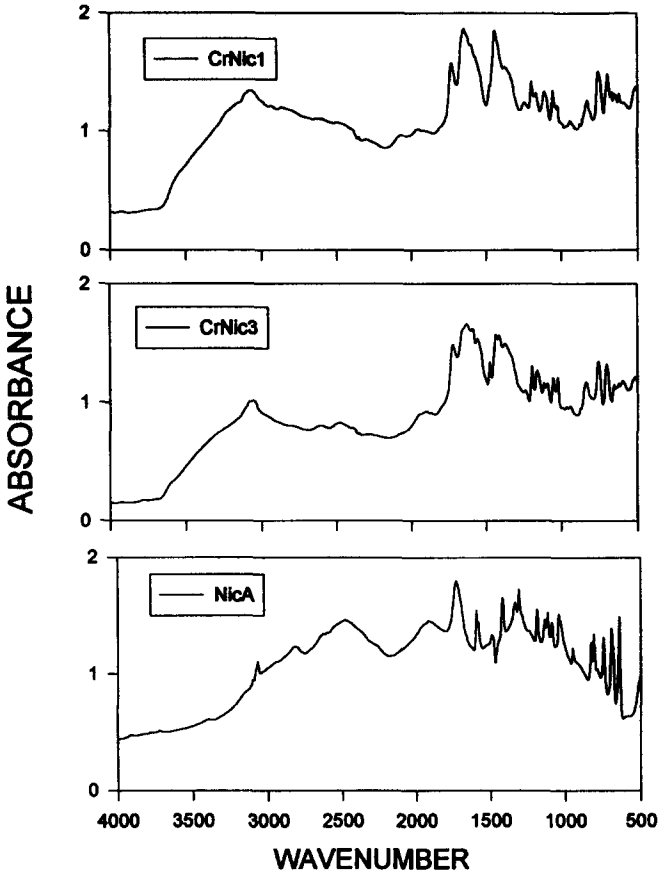

Figure 9. FTIR diffuse reflectance spectra. (a) Upper curve: CrNic1. (b) Middle curve: CrNic3. (c) Lower curve: NicA.

Discussion

The NMR spectra, molecular modeling, and IR spectroscopy concur that CrPic is a strongly bonded complex, and are in agreement with a bidentate meridional tripicolinate crystal structure [10]. The red color is indicative of Cr–N bonding. Since no ^{1}H or ^{13}C signal was observed, the signal for the CrPic molecule is so broadened, or shifted so far down field, that it is not detectable. Very strong downfield shifting of the deuterium NMR spectra resulting from Cr–N bonding was also observed by Green et al. [13]. The *trans*-$[Cr(1,3\text{-pn})_2(nic)_2]Cl \cdot 4H_2O$ complex described in the Introduction, as well as three similar complexes stable only in very acid solution (pH 0.2–1.3; pyridine N protonated), had $\delta + 0.1$ to $+6.7$ with respect to deuterated methyl trichloride at $+7.2$. In contrast, Cr methyl nicotinate (in EtOH) and Cr malonate (in MeOH) complexes with coordination via the pyridyl N had a dramatic δ of -70. In general, shifts downfield indicate electron density donation, and shifts upfield indicate electron acceptance. With this criterion, Cr is a very strong net donor to the CrPic complex.

Our lowest energy structures for PicA have the OH group nearest N as opposed to =O, which indicates that the bidentate ligand bonds via N and OH, not N and =O. This information is not available from the x-ray diffraction data. This structure is consistent with CrPic's

high stability in acid solution, poor H_2O solubility, and the destruction of the complex by strong base.

As indicated above, Cr(III) is represented in the molecular structures by a spherically symmetric point charge. The actual Cr(III) ion has $4s^2 3d^3$ electronic configuration, which results in two axial and four equatorial bonding orbitals, and typically octahedral site symmetry. In the meridional isomer structure, only two of the three orthogonal PicA ligands can be in identical environments, which results in an asymmetrical chiral molecule.

The green and blue colors of the CrNic complexes are indicative of O bonding as opposed to N or bidentately. However, our NMR data do not support significant bonding to the COOH group, as proposed for other complexes [12–16]. In particular, CrNic2 and CrNic3 show no evidence of permanent coordination with Cr. Nonspecific peak broadening (i.e., loss of splitting) is caused by the strong paramagnetism of transition metals such as Cr, and can be present regardless of whether the metal is coordinated to a molecule, or simply present in the sample in an ionic or free metallic form. The molecular structures with N-coordination of NicA were also more stable than the O coordinated structures.

Peaks 5, 6, 7, and 8 in CrNic1 (Fig. 3a) show evidence of stronger complexing than peaks 1, 2, 3, 4; however, the former peaks are shifted greatly downfield, while the latter are shifted slightly upfield with respect to NicA. There are at least two entirely different magnetic environments for *all* the protons in this molecule. The strong shift upfield indicates that Cr is a net electron acceptor with respect to NicA, not a strong donor as in CrPic above. In addition, the ^{13}C data show uniform, minor chemical shifts for CrNic1 as compared to free NicA. If there were strong, localized bonding to the COOH group, then C1 should have shown a greater shift than C2–C6 on the pyridine ring. Green et al. [13] reported a $\delta = -77.2$ for a *trans*-N bonded complex and $\delta = +0.5$ to 1.9 for *trans*-O bonded complexes with deuterium NMR; however, this does not completely rule out some limited Cr–N bonding in CrNic1. The relative degree of chemical shift for ^1H on our instrument would be $\sim 15\%$ of the Green et al. values, or circa 11.9 for N bonding and 0.038–0.3 for O bonding. Our chemical shifts with respect to the peak positions of free NicA are circa 2.0, which is intermediate between these values. We note also that limited Cr–N bonding is supported by the ^{13}C and the exchange data (discussed below). When CrNic1 and CrPic were mixed, the data indicated that at least some NicA molecules were rotating around the pyridine nitrogen. While our data do not allow us to propose a bidentate or *trans*-N configuration for CrNic1, it is possible that Cr in this complex is bonded to a number of OH or H_2O molecules, and occasionally to NicA via one Cr–N bond.

Both the lack of crystallinity of all the CrNic complexes and of evidence for Cr–COOH or extensive Cr–N bonding indicate that these are polymeric, hydrated complexes. Cr(III) ions and nicotinic acid molecules are in weak, nonstochiometric association with numerous OH and/or H_2O molecules. The IR spectra confirm this since CrNic1, 2, and 3 (Figs. 8, 9) have the requisite characteristic broad absorption bands centered around 3000 cm^{-1}. This type of structure is consistent with the observations that CrNic2 and CrNic3 are highly insoluble in both water and DMSO, and have little or no complexing apparent in the NMR spectrum. However, it is important to recognize that the IR spectra of CrNic1, 2 and 3 hardly differ, but their colors, solubilities, and NMR spectra do. This indicates that IR diffuse reflectance spectroscopy is not a sufficient analytical technique to detect the degree of complexing between Cr and NicA.

The ^1H NMR data for the CrPic/CrNic1 mixtures show that exchange between these molecules occurred in DMSO solution. The behavior of peaks 1 and 2 in Fig. 6 indicates that these proton environments are changing at a rate on the order of the NMR experimental time frame. In the pure CrNic1 solution, 1 and 2 occur as sharp, distinct peaks. In the CrPic/CrNic mixed solutions, these peaks broaden, and tend to coalesce. At the ratio 4/12 CrPic, peaks 1 and 2 are not distinguishable, indicating that these two proton environments are in effect identical at that CrPic/CrNic1 ratio. Other ratios not shown also had complete coalescence; however, uniform behavior of the spectra with respect to the CrPic/CrNic1 ratio cannot be quantified because the MW of CrNic1 is unknown.

Peaks 1 and 2 (Fig. 3a) are in the set of CrNic1 peaks that have δ values close to free NicA, and represent the protons on either side of the pyridine N. We hypothesize that in the mixed solution, exchange can result in a molecule with one Cr bonded to two PicA and one NicA. This type of molecule is conceptualized in Fig. 7b. Cr–N bonding is proposed, and rotation of the pyridine ring around this bond can account for the observation that the proton environments 1 and 2 become indistinguishable. This conformation also has a lower energy than the conformation where one NicA is bonded via O (Table 3). The ^{13}C data support this type of exchange. Figure 5 shows small uniform chemical shifts, which do not support localized bonding via the carboxyl group. The ^{13}C CrPic/CrNic1 mixtures showed similar uniform chemical shifts, and thus did not indicate that exchange involved localized COOH bonding.

Figure 7 is based on exchange of one NicA molecule for one PicA molecule starting with a $CrPic_3$ molecule. As we have noted, the actual Cr coordination sphere is expected to be octahedral with two axial and four equatorial ligand bonds. For example, if two PicA pyridyl N are coordinated to two axial sites, then the third must be coordinated to an equatorial site. This different third site may be the locus of exchange, and the equilibrium constant for exchange on that site may be relatively favorable. In this case, we would expect the equilibrium constant for the *next* exchange (i.e., $CrPic_2Nic \leftrightarrow Cr\text{-}PicNic_2$) to be less favorable. We also realize that while the spectral data clearly indicate that some type of exchange is occurring, the exchange process and the resultant structures may be very complex, especially since the "structures" of the CrNic complexes are not simple.

The exchange of one NicA for PicA (Fig. 7b) can serve as a model for further investigations. If, instead of NicA, a peptide or protein exchanged with the PicA, this could provide part of the mechanism for the absorption and active transport of CrPic complex across cell membranes. CrPic has been shown to be an efficacious Cr nutritional supplement [5, 6, 9], and this is an area for future research.

Conclusion

It is not apparent from our data that NicA is oxygen coordinated to Cr in any of three different CrNicA complexes. The green color indicative of O coordination may be due wholly or partly to O coordination with H_2O and/or OH, not NicA. This is in agreement with Green et al. [12] and Cooper et al. [14], who found that Cr *trans*-$NicA_2$ complexes were stabilized by the axial ligands 1,3-pn, H_2O, or amino acids, not by NicA. CrNic1 has one set of peaks with a strong chemical shift indicating that at least some of the NicA may be more strongly associated with Cr. The magnitude of this shift coupled with the ^{13}C and the exchange data suggest that limited Cr–N bonding may occur in CrNic1.

CrNic2 and CrNi3 do not have two set of peaks, and show little difference from the free NicA spectra. The CrNic2 and CrNic3 spectra are, in fact, similar to Fig. 2b, where the addition of a strong base destabilized and eventually destroyed the CrPic complex. The FTIR spectra of all CrNic complexes show considerable bound OH and/or H_2O. CrNic complexes in general are likely to be olates, with Cr and NicA held loosely in a high molecular weight OH polymer. An olate structure is particularly consistent with the observation that CrNic2 and CrNic3 are relatively insoluble in H_2O and DMSO, and can explain the blue and green colors observed.

An olate structure for CrNic indicates that this form of Cr supplementation may not be well absorbed, especially considering that Cr is exchange neutral [18]. Nicotinic acid as well as Cr appear to be necessary for normal insulin functioning [19]; however, the nicotinic acid in a typical 100–400 mcg Cr nicotinate supplement is not quantitatively significant for nicotinic acid-deficient individuals. In contrast, CrPic is a very stable strongly bonded complex. Although it is stable, it does readily exchange with CrNic1 in DMSO solution. This exchange may provide a mechanism for the absorption and active transport of Cr in biological systems.

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